DIVISION S-4—SOIL FERTILITY & PLANT NUTRITION

Nitrogen Budget and Soil N Dynamics after Multiple Applications of Unlabeled or ^{15}Nitrogen-Enriched Dairy Manure

Gabriela R. Muñoz,* J. Mark Powell, and Keith A. Kelling

ABSTRACT

Repeated N applications to field crops, either as inorganic fertilizers or animal manures, can lead to N buildup in soils with potential long-term environmental hazards. The objective of this 3-yr field study was to monitor total- and mineral-N levels in soil after repeated fertilizer or single or repeated dairy manure applications, and to compute an N balance for the soil-crop system. Unlabeled and ^{15}N-enriched dairy manure were used. The experiment was conducted on a Plano corn silt loam continuously cropped to corn (*Zea mays* L.) Manure increased total- and NO_3–N levels in soil, especially in the 0- to 30-cm depth and in plots receiving frequent and recent manure applications. Manure increased NO_3–N in the 0- to 30-cm soil layer more than fertilizer N, whereas the opposite was true in the 30- to 60- and 60- to 90-cm layers. There was a clear NO_3–N buildup with repeated manure treatments. Unlabeled N measurements were not accurate enough to track trends in soil total N levels, hampering the calculation of an N balance. ^{15}Nitrogen-labeled manure allowed for direct measurement and provided more accurate estimates of N recovery in soils and crops. During the 3-yr study period, an average of 18% of applied manure ^{15}N was recovered in corn silage and 46% remained in the soil. Unaccounted-for ^{15}N (36%) was assumed to be lost mainly by NH_3 volatilization and denitrification. Most (82%) of the ^{15}N remaining in soil was present in the top 30 cm, irrespective of frequency of manure application. Although costly and time-consuming, the use of ^{15}N-labeled manure provided a much better approach to study the fate of manure N within the soil-crop system, compared with unlabeled manure.

In most agricultural soils, N is the most limiting nutrient, and it has to be supplied to cereal crops, particularly in high productivity systems (Meisinger, 1984). Fertilizers, manure, and in some cases, legumes, are the principal N sources for crop production in mixed, dairy-crop production systems. Whereas fertilizer N is readily soluble in soils and becomes immediately available for crop uptake, it can also be highly susceptible to leaching losses. Comfort et al. (1987) found that soil inorganic N and downward movement were increased to a greater extent by fertilizer than by manure N. On the other hand, only about half of manure N is inorganic, with the rest being present in organic forms. Organic N must be mineralized before it can be used by plants or it becomes susceptible to losses. However, when fresh manure or slurry contain appreciable amounts of urea or NH_4, N can be easily lost via NH_3 volatilization, especially if manure is surface-applied (Thompson et al., 1987). Conversely, denitrification losses of manure N are increased by incorporation or injection (Comfort et al., 1988), and because organic matter in manure serves as a substrate for denitrifier microorganisms, tend to be higher than denitrification losses in fertilized soils (Cates and Keeney, 1987). Immobilization of inorganic N in manure, plus the greater volatilization and denitrification losses, cause inorganic N in manure to be less plant-available than equal rates of inorganic fertilizer (Paul and Beauchamp, 1993). Because of its lower N availability, greater amounts of manure than fertilizer N are applied to crops. This can result in a steady accumulation of soil N. Long-term soil N accumulation on dairy farms poses a serious environmental risk (Bouldin et al., 1984). Heavy and/or repeated manure applications can lead to NO_3–N buildup in soil and losses through leaching (Adriano et al., 1971; Mathers and Stewart, 1974; Smith et al., 1980; Cooper et al., 1984). Dairy farms in the Midwest are considered significant contributors of N to the hypoxic zone in the Gulf of Mexico (Burkart and James, 1999).

Excessive soil nutrient accumulation and losses to surface and ground water are pressing environmental challenges facing the dairy and other animal industries. As dairy herds expand to remain economically viable, a larger percentage of the available cropland is devoted to corn silage. The noted expansion of corn silage production (Battaglia, 1999; Shaver, 2000) is due to this crop's ability to feed more cows (*Bos taurus*) than other forages per unit of cultivated area (Seglar, 1998), as well as favorable economics (Klemme, 1998) to the farmer. However, the effects of shifting more land to corn silage on other system components, such as N use, buildup and loss remains to be determined. Since only a relatively small amount of applied N is ultimately taken up by the crop, we wanted to track the fate of the unused portion to see whether it was lost or remained in the soil.

The objective of this study was to determine total and inorganic soil N and the N balance of a continuous corn silage cropping system receiving two fertilizer or dairy manure N rates of different application frequency across 3 yr. Unlabeled and ^{15}N-enriched dairy manure were used, and the ability of each manure type to detect trends in soil N levels and account for applied N was compared. The use of ^{15}N-labeled manure was an essential part of the study because it allowed direct N tracking in the cropping system, and provided more accurate measurements than unlabeled manure.

G.R. Muñoz and K.A. Kelling, Dep. of Soil Science, Univ. of Wisconsin, 1525 Observatory Dr., Madison, WI 53706; and J. Mark Powell, USDA-ARS Dairy Forage Research Ctr., 1925 Linden Dr. West, Madison, WI 53706. Received 22 Feb. 2002. *Corresponding author (grmunoz@uwalumni.com).

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MATERIALS AND METHODS

Field Experiment

A field trial was conducted from 1998 to 2000 at the West Madison Agricultural Research Station in Madison, WI (45°05′ N, 89°31′ W) on a Plano silt loam (fine-silty, mixed, superactive, mesic, Typic Argiudolls). Initial surface (0 to 15 cm) soil tests values were: pH 6.7 (water); organic matter, 41 g kg^{-1} (loss on ignition); Bray P_1 and K levels of 50 and 146 mg kg^{-1}, respectively. Total-, NH_4–, and NO_3–N levels in the upper 30 cm of soil were 2026, 14, and 8.2 mg kg^{-1}, respectively.

Before experiment initiation, the field was cropped to alfalfa (*Medicago sativa* L.) from 1994 to 1996 and to corn in 1997. No manure had been applied for at least 4 yr before the start of the trial. Treatments consisted of two inorganic fertilizer N levels (90 or 179 kg ha^{-1}, as NH_4NO_3), two manure rates (estimated to provide ≈90 and 180 kg available N ha^{-1} to corn the first year following application), a control receiving neither fertilizer nor manure, and three manure application intervals (every 1, 2, or 3 yr). The same manure N rate was used every year it was applied. Fertilizer N was applied every year to the same plots. The design of the field trial was a split plot, with fertilizer treatments and manure rates applied to whole plots. Application intervals were the subplots within the manure whole plots. Nitrogen rates were applied to subplots in the fertilizer whole plots. Whole and subplots (henceforth referred to as plots) were arranged in four randomized-complete blocks to give four replications of each treatment. The plots were 10.6 by 6 m, separated by 1.5-m alleys and contained eight corn rows that were 0.75 m apart. For the ^{15}N experiment, microplots of 1.5 by 2.3 m containing three corn rows were established within each of the low manure rate plots, following the design proposed by Jokela and Randall (1987). Manure applied to these microplots in 1998, 1999, and 2000 had an atom % ^{15}N of 1.47, 1.12, and 1.64, respectively.

Fresh dairy manure (composite of feces, urine, and straw bedding) was collected from a stockpile. Manure for the ^{15}N microplots was labeled by feeding cows ^{15}N-enriched silage and alfalfa following the procedure described by Powell and Wu (1999) to obtain labeled urine and feces having uniformly-labeled microbial and undigested feed N components. Fertilizer and manure were applied ≈5 d before planting. The field was disked twice within 3 to 20 h after manure application. Manure amounts and manure N and NH_4–N applied each year are presented in Table 1. Manure dry matter content was 210, 260, and 240 g kg^{-1} for 1999 through 2000, respectively. Manure enriched with ^{15}N had a dry matter content of 170 g kg^{-1} all 3 yr. Corn (cv. Lemke 6063) was planted every spring. Starter fertilizer (9-23-30, 224 kg ha^{-1} in 1998 and 1999, and 168 kg ha^{-1} in 2000) was band-applied to all plots at planting.

Corn aboveground tissue was harvested at approximately physiological maturity from plots and microplots separately.

Table 1. Manure and manure N applied to the experimental plots in south-central Wisconsin, 1998 to 2000.

	Applied manure†			Total N applied			NH_4–N applied	
Year	^{15}N	L‡	H‡	^{15}N	L	H	L	H
	Mg ha^{-1}			kg ha^{-1}				
1998	63	35	70	224	194	388	57	113
1999	63	38	77	284	250	501	69	137
2000	63	34	68	235	233	489	83	171
Mean§	63	36	72	248	226	459	70	140

† Wet weight.
‡ Unlabeled manure Low and High rates.
§ Across years.

After sampling, the remaining plants were removed from the field. The site was chisel plowed each fall. Soil samples were taken using a stainless steel auger to 90 cm in 30-cm increments each spring, before fertilizer and manure application, and each fall, about a week after harvest. At the beginning of the experiment (May 1998), composited samples from 12 cores were taken from two locations per block. Thereafter, samples (four-core random composite) were retrieved from the control and fertilized plots, and from plots receiving manure every year at both application rates. Subsamples were oven-dried (60°C), ground to pass a 2-mm sieve, and analyzed for total-, NH_4–, and NO_3–N.

In fall 2000, all ^{15}N microplots were systematically sampled 25 cm apart from the center of the microplot in every direction (i.e., two cores from the central row and two cores from between rows) using a plywood template. Samples were processed and analyzed similarly to those from the main plots. In addition, ^{15}N enrichment of soil total- and inorganic-N fractions was determined. For ^{15}N enrichment analysis, soil subsamples were hand-ground in a ceramic mortar and sieved to pass a 100-μm mesh screen.

Chemical Analyses

Manure N content was analyzed following the procedures outlined by Combs et al. (2001). Total soil N was determined following a Kjeldahl digestion (Nelson and Sommers, 1972) with these modifications: 1.5 g of Kjeldahl mix (Na_2SO_4–$CuSO_4$–Se in a ratio of 1000:32:5) and 5 mL concentrated H_2SO_4 were used. Our soil typically contained <1% of total N as NO_3–N, therefore, a Kjeldahl digestion provided an adequate measure of total N (Bremner, 1996). For the topsoil (0 to 30 cm), a 1-g subsample was digested, and a 2-g subsample was used for soil taken from lower (30- to 60- and 60- to 90-cm) depths. The digest was diluted to 50 mL, filtered through acid-washed Whatman no. 2 filter paper and analyzed for NH_4–N in an automated colorimeter (Lachat Instruments, Milwaukee, WI) using the QuikChem Method 13-107-06-2-D (Lachat Instruments, 1992b), with sodium phenate, and 5.2% sodium hypochlorite. Total N in plant tissue was determined following a similar procedure on 250-mg samples.

Soil NH_4– and NO_3–N were determined according to a modification of the procedure described by Liegel et al. (1980). The KCl extract was filtered through Whatman no. 2 paper and analyzed for NH_4–N following the same procedure already described, and for NO_3–N using the QuikChem Method 12-107-04-1-B (Lachat Instruments, 1992a).

For soil inorganic (NO_3 plus NH_4) ^{15}N enrichment, KCl extracts were treated following the microdiffusion technique described by Stark and Hart (1996). Total N and ^{15}N concentrations in soil, corn tissue, and manure samples from ^{15}N microplots were determined using a Carlo Erba (Milan, Italy) elemental analyzer coupled with a mass spectrometer Europa 20/20 tracermass.

Statistical Analyses

Statistical analyses were performed using SAS software (SAS Institute, 1990). When there were soil N measurements repeated in time, ANOVA was conducted across sampling times and treatments. Given the differences known to exist from one cropping season to the next, which were usually reflected in the statistical analyses, ANOVA was performed by sampling time as well, to distinguish between treatments. Depth was acknowledged to have a major influence on soil characteristics and hence N levels; therefore, ANOVA was performed by depth. Polynomial models were fitted to soil N levels vs. sampling time (each time was assigned a number from 1 to 6), and the best fit was chosen between first- and second-order polynomials.

Calculations

Cumulative N recovery in corn was calculated by the difference method:

$$\text{Cumul N recov \%} = \frac{\Sigma(\text{Tmt N upt} - \text{Ctrl N upt})}{\Sigma \text{ applied N}} \times 100 \qquad [1]$$

where Tmt N upt and Ctrl N upt is the N (kg ha^{-1}) contained in the aboveground dry matter for a given treatment and control plots, respectively. Applied N is the total amount of N applied as fertilizer or manure, and the summations are across years.

Total soil N to a depth of 90 cm, harvested whole-plant N uptake, and manure N additions were used to compute soil–crop N balances. Nitrogen recovered in the soil (% N recov_{soil}) after 3 yr of fertilizer or manure applications was calculated according to:

$$\% \text{ N recov}_{soil} = \frac{(\text{soil N}_{tmt,f} - \text{soil N}_{tmt,i}) - (\text{soil N}_{ctrl,f} - \text{soil N}_{cntrl,i})}{\Sigma \text{ applied N}} \times 100 \qquad [2]$$

where soil N is the total-N in soil for treatment and control plots (tmt and ctrl) at the beginning and end (i and f) of the 3-yr study period, and the summation is across years. The soil N values were derived from regression curves for total soil N vs. sampling time. Hence, total soil N in the control treatment remained the same throughout the period (Fig. 1) and the second term in Eq. [2] was set to zero.

Cumulative recovery of ^{15}N in corn was calculated according to the following equation, adapted from Hauck and Bremner (1976):

$$\text{Cumul } ^{15}\text{N recov \%} = \frac{\Sigma p(c - d)}{\Sigma k(a - b)} \times 100 \qquad [3]$$

where p = total N in corn, k = total N applied with manure, a = atom % ^{15}N of manure applied, b = atom % ^{15}N in unlabeled manure (0.377 in 1998, and 0.366 in 1999 and 2000), c = atom % ^{15}N of corn, d = atom % ^{15}N in control corn, and the summation is across years.

Similarly, recovery of ^{15}N in soil was calculated as:

$$^{15}\text{N recov \%} = \frac{q(e - g)}{k(a - b)} \times 100 \qquad [4]$$

where q = total N in soil, e = atom % ^{15}N of soils, g = atom % ^{15}N in control soils (0.366).

Total N recovered in soil plus harvested corn was calculated for both N and ^{15}N as:

$$\text{Tot N recov \%} = \% \text{ N recov}_{soil} + \% \text{ N recov}_{harv\ corn} \qquad [5]$$

Nitrogen losses were not directly measured in this experiment. Unaccounted for N was assumed to be lost by NH_3 volatilization, denitrification, and/or leaching (mainly between cropping seasons). Where appropriate, soil total- or NO_3–N concentrations in mg kg^{-1} were converted to kg ha^{-1} by assuming a soil bulk density of 1.3 g cm^{-3}.

An estimation of soil NO_3–N changes due to manure applications was obtained by monitoring soil ^{15}N concentrations in the inorganic-N (NH_4 plus NO_3) fraction in subplots amended with ^{15}N-labeled manure. For these calculations, ^{15}N enrichment was assumed to be similar for NH_4– and NO_3–N, and therefore equal to that of the inorganic fraction. Soil $^{15}NO_3$–N increase was entirely due to manure, and it equaled the $^{15}NO_3$–N excess (over natural abundance) measured after applications, since initial ^{15}N excess was zero. Percentage ^{15}N recovered in soil by the end of the third year as $^{15}NO_3$–N was calculated in a manner similar to Eq. [3] and [4]:

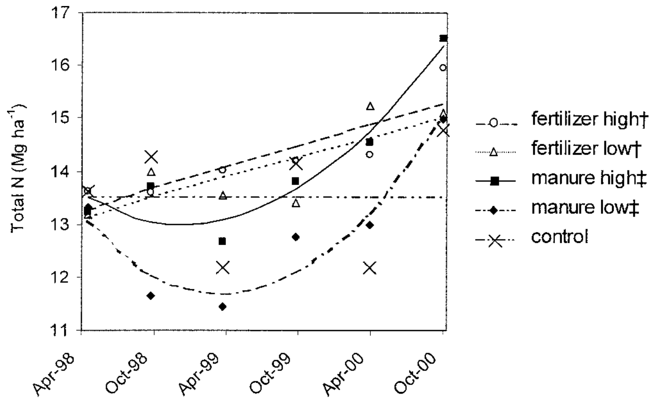

Fig. 1. Actual and fitted total soil N levels to a depth of 90 cm as affected by repeated manure or fertilizer applications in south-central Wisconsin, 1998–2000. †Fertilizer rates were 90 and 179 kg N ha^{-1} yr^{-1} for the low and high levels, respectively. ‡3-yr average manure rates were 236 and 459 kg total N ha^{-1} yr^{-1} for the low and high levels, respectively. Regression equations: Fertilizer high, $y = 12.88 + 0.4004x$, $R^2 = 0.743$, $P = 0.027$; Fertilizer low, $y = 12.76 + 0.3775x$, $R^2 = 0.637$, $P = 0.057$; Manure high, $y = 14.48 - 1.241x + 0.2595x^2$, $R^2 = 0.912$, $P = 0.026$; Manure low, $y = 14.83 - 2.115x + 0.3579x^2$, $R^2 = 0.906$, $P = 0.029$; Control, $y = 13.53$.

$$^{15}\text{NO}_3\text{–N recov \%} = \frac{\text{NO}_3\text{–N}_f(j - l)}{\Sigma[k(a - b)]} \times 100 \qquad [6]$$

where NO_3–N_f is the final soil NO_3–N concentration (i.e., measured in the fall of 2000), i and l are atom% inorganic ^{15}N of treated and control soil, respectively, and the summation is across years. The amount of total NO_3–N (both ^{14}N and ^{15}N) originating from manure was obtained by multiplying Eq. [6] by total applied N. An equivalent calculation was made by replacing NO_3–N concentrations by total-N and using soil ^{15}N enrichments measured in that fraction.

RESULTS AND DISCUSSION

Inorganic Soil Nitrogen

Measured soil NH_4–N levels for all sampling times and depths were between 5 and 15 mg kg^{-1} and no significant differences could be attributed to treatment, and are therefore not presented. Similarly, Chang et al. (1991) found no significant effect of repeated manure applications (for 11 consecutive years at rates as high as 180 Mg ha^{-1} yr^{-1}), on soil NH_4–N levels at any depth.

Soil NO_3–N levels at each sampling depth and time are presented in Table 2 for the controls and plots receiving annual inorganic fertilizer or manure applications. Statistical analyses were done on each soil layer separately. There were significant effects of treatment, time, and treatment × time on soil NO_3–N levels at all depths, except for the 30- to 60-cm layer, where the interaction was not significant. Probability values were <0.001 in

Table 2. Effect of repeated annual manure and fertilizer applications on spring and fall soil NO_3–N levels in south-central Wisconsin, 1998 to 2000.

Treatment	1998 Spring	1998 Fall	1999 Spring	1999 Fall	2000 Spring	2000 Fall
	mg kg^{-1}					
			Depth 0 to 30 cm			
Fertilizer high†	8.0	6.7	5.9	11.0	11.0	11.1
Fertilizer low†	9.4	6.5	5.2	7.3	11.8	9.2
Manure high‡	7.5	4.2	5.1	13.7	15.2	18.3
Manure low‡	6.8	5.9	6.0	8.8	11.6	10.8
Control	8.0	4.9	6.2	6.1	9.6	6.4
P value	0.699	0.175	0.620	0.001	0.001	<0.001
LSD	ns§	ns	ns	3.1	2.1	2.2
			Depth 30 to 60 cm			
Fertilizer high	6.1	7.1	7.5	4.4	10.5	5.0
Fertilizer low	5.5	2.5	8.2	1.5	7.0	4.8
Manure high	3.3	2.8	7.5	1.9	9.7	3.6
Manure low	3.2	2.1	5.4	1.0	6.0	2.3
Control	6.1	1.9	5.7	1.0	5.2	1.3
P value	0.345	0.073	0.128	0.010	0.003	0.013
LSD	ns	2.2	ns	1.9	2.6	2.1
			Depth 60 to 90 cm			
Fertilizer high	5.5	6.6	7.4	4.7	8.5	6.7
Fertilizer low	2.9	1.1	6.5	3.4	4.7	4.0
Manure high	3.7	0.9	7.6	2.4	6.3	4.1
Manure low	5.2	1.8	5.3	2.2	2.7	1.8
Control	5.5	1.2	6.8	1.2	2.7	0.7
P value	0.395	<0.001	0.356	0.014	<0.001	0.037
LSD	ns	2.0	ns	1.9	2.2	3.5

† Fertilizer rates were 90 and 179 kg N ha^{-1} yr^{-1} for the low and high levels, respectively.
‡ Three-year average manure rates were 236 and 459 kg total N ha^{-1} yr^{-1} for the low and high levels, respectively.
§ Not significant.

all cases except for treatment × time at the deepest layer ($P = 0.02$). Statistical analyses of soil NO_3–N levels by time and depth (Table 2) showed significant differences among treatments in the topsoil (0 to 30 cm) after the second cropping season (i.e., after two fertilizer or manure applications); hence, the following observations refer to those sampling times. The lowest and highest NO_3–N levels always corresponded to the control and the high manure rate, respectively. Differences tended to become more pronounced with time. There was usually no difference in soil NO_3–N levels in plots amended with the low manure rate or either fertilizer rate. Manure at the high rate significantly increased NO_3–N compared with the low rate and the control.

In the 30- to 60-cm soil layer, significant differences were found at the same sampling times as for the topsoil and, in addition, after the first application (fall of 1998). In all of these instances, the high fertilizer rate significantly increased NO_3–N levels with respect to control plots, which had the lowest NO_3–N concentrations. Manure applied at the high rate resulted in significantly higher NO_3–N levels than the control in 2000 only. The low manure rate, on the other hand, had NO_3–N levels in the 30- to 60-cm soil layer that were never statistically different from the control. Fertilizer applied at the low rate gave similar NO_3–N levels as manure applied at the high rate, except in spring 2000, when it was significantly lower. Greater downward NO_3–N movement was also found for fertilizer than manure applications by other authors (Sutton et al., 1978; Comfort et al., 1987).

Significant differences in soil NO_3–N concentrations at the 60- to 90-cm depth were also detected after the first cropping season, except for spring 1999. Plots that received fertilizer at the high rate had significantly higher NO_3–N levels than the control, which always had the lowest values. Soil NO_3–N in plots receiving the high manure rate was higher than the controls in spring 2000 only. Plots fertilized at the low rate had higher NO_3–N levels than the control in fall 1999.

Both fertilizer and manure additions tended to increase soil NO_3–N compared with the control. Although the fertilizer effect on topsoil NO_3–N levels was lower than that of manure, fertilizer increased NO_3–N concentrations in lower soil depths indicating that more fertilizer- than manure-N moved downward as NO_3–N during the growing season. This difference in behavior was probably due to the fact that NO_3–N applied as inorganic fertilizer is immediately solubilized in soil and therefore more susceptible to downward movement within the soil profile if there is no crop to utilize it. More than half of manure N, on the other hand, is in organic forms, and virtually all of the rest is present initially as NH_4–N. Hence, manure organic N has to be mineralized and nitrified before it becomes susceptible to leaching.

Another interesting trend observed in Table 2 is the almost invariably higher NO_3–N concentrations observed in the spring than in the fall, at the 30- to 60- and 60- to 90-cm soil depths. This was likely due to downward movement of some N during the previous winter and early spring, whereas the fall sampling reflects a decrease in the NO_3–N pool during the cropping season by plant uptake, and perhaps leaching; however, previous research on similar soils has shown little leaching during the growing season (Olsen et al., 1970; Kelling

Table 3. Regression equations used to describe soil NO_3–N levels to the 30-cm depth across time after repeated manure and fertilizer N applications in south-central Wisconsin, 1998 to 2000.

Treatment	Regression equation†	R^2	P value
Fertilizer high‡	$y = 5.7 + 0.95x$	0.270	0.011
Fertilizer low‡	$y = 8.1$	–	–
Manure high§	$y = 1.4 + 2.7x$	0.605	<0.001
Manure low§	$y = 4.4 + 1.1x$	0.407	0.001
Control	$y = 6.9$	–	–

† y is the soil NO_3–N concentration in mg kg^{-1}, x is the sampling time (1–6 corresponding to spring, 1998, through fall, 2000, as shown in Table 4).
‡ Fertilizer rates were 90 and 179 kg N ha^{-1} yr^{-1} for the low and high levels, respectively.
§ Three-year average manure rates were 236 and 459 kg total N ha^{-1} yr^{-1} for the low and high levels, respectively.

et al., 1977). Topsoil (0 to 30 cm) NO_3–N concentrations did not follow the same pattern as at lower soil depths, probably because of continual mineralization and subsequent nitrification in the upper soil profile.

There was a clear trend, described by linear regression, toward increased NO_3–N concentrations across time in the topsoil for both manure rates and for the high fertilizer rate. Equations and statistics are presented in Table 3. Regressions were not significant for the deeper soil layers. The slope for the high manure rate was significantly greater than those of the other treatments ($P < 0.0001$), indicating that NO_3–N tended to accumulate to a greater degree in manured than fertilized plots. This also appears to support the argument for greater short-term leaching potential from the fertilizer. Comfort et al. (1987) found that high manure rates did not significantly increase inorganic N below 30 cm in the first application year, whereas fertilizer N did, and Kimble et al. (1972) measured more NO_3–N available for leaching in fertilized than manured plots. Jokela (1992) found that manure had the same, or slightly lower leaching potential than an agronomically equivalent fertilizer rate.

It might be expected that the downward movement of NO_3–N will eventually become a problem even for manure, especially at high manure rates. Chang et al. (1991) reported increases of 0.72 Mg NO_3–N ha^{-1} after 11 yr of continuous manure application at a rate of 30 Mg manure ha^{-1} yr^{-1} (wet weight), with effects extending to the 150-cm depth. Nitrate in the 0- to 150-cm depth continually increased with repeated manure applications (20 yr) in nonirrigated sites (Chang and Janzen, 1996). After three annual manure applications, Comfort et al. (1987) reported similar soil NO_3–N for fertilizer and manure treatments that were usually significantly higher than controls at the 60- to 120-cm depth after harvest.

Total Soil Nitrogen

Total soil N concentrations by depth at each of the six sampling times are shown in Table 4. As would be expected, total N concentrations decreased with soil depth. Regression analyses were performed to describe the effect of repeated N applications on total soil N (Mg ha^{-1}) across time for the 0- to 90-cm depth. Soil N levels in control plots did not change during the 3-yr study period ($P = 0.89$), averaging 13.5 Mg ha^{-1}. Fertilizer and manure treatments significantly increased total soil N during the study. Soil N increases due to fertilizer were best described by linear regression, whereas those due to manure were best described by second order polynomials. Regression equations, statistics, and graphics are presented in Fig. 1.

Net total soil N increases in plots that received the low and high manure rates, based on regression analyses, were 2.0 and 2.9 Mg ha^{-1}, respectively, across the 3-yr study period. For fertilizer, these values were 1.9 and 2.0 Mg ha^{-1}. It is clear that these measurements are not sufficiently accurate, since they predict soil N increases much higher than the total N applied (0.7 and 1.4 Mg ha^{-1} for manure, and 0.3 and 0.5 Mg ha^{-1} for fertilizer). It should be noted that, for most treatments, the last soil sample taken showed much higher N levels than the previous five. This greatly influenced our estimation of total soil N at the end of the third study year. If the fall 2000 soil samples were anomalously high, it is possible that additional observations may rectify our estimations.

Use of ^{15}Nitrogen-labeled Manure

Soil Nitrate-Nitrogen

According to soil $^{15}NO_3$–N measurements, soil NO_3–N increases due to 3 yr of manure application ranged from 0.1 to 6.7 kg ha^{-1} (Fig. 2). Most (74 to 94%) of this NO_3–N was found in the upper 30 cm of soil. On average, more NO_3–N was found in the 60- to 90-cm than in the 30- to 60-cm depth, but the difference was not significant and was likely due to high variability and/or the movement of N from previous year applications. Statistical analyses revealed that manure application interval had a significant effect on NO_3 levels both in the 0- to 30-cm depth and the entire 0- to 90-cm sampled depth (P = 0.006 and 0.060, respectively). The greatest amount of soil NO_3–N was found in plots receiving more frequent or recent manure applications (Fig. 2). This likely reflected the higher N loads resulting from repeated manure applications, and higher crop uptake and losses from manure that remained in the soil for a longer period. Treatment did not have an effect on $^{15}NO_3$–N in the 30- to 60-cm depth. The only significant difference found at the 60- to 90-cm layer was for plots continuously manured, which had concentrations significantly higher than any other treatment. Manure increased NO_3–N levels in the subsoil only in plots that received three consecutive manure applications, and only to a small degree. These data confirmed the large plot trends (Table 2), that manure applied at the low rate had a low leaching potential during this 3-yr period. Other researchers conducting direct ^{15}N leaching measurements from manure showed leaching losses that ranged from <0.3 to 4% after 2 yr, with most of the leaching occurring during the year of application (Sørensen et al., 1994; Sørensen and Jensen, 1998). However, NO_3–N leaching is likely to be underestimated by ^{15}N experiments. Sørensen et al. (1994) measured much higher

Table 4. Effect of repeated annual manure and fertilizer applications on spring and fall total soil N levels in south-central Wisconsin, 1998 to 2000.

Treatment	1998 Spring	1998 Fall	1999 Spring	1999 Fall	2000 Spring	2000 Fall
	mg kg^{-1}					
			Depth 0 to 30 cm			
Fertilizer high†	1954	2079	2002	2144	2102	2361
Fertilizer low†	2073	2117	2038	2045	2264	2277
Manure high‡	1900	2074	1924	2043	2135	2464
Manure low‡	1944	1792	1755	1855	1916	2252
Control	1954	2271	1467	2096	1572	2115
P value	0.112	0.319	0.105	0.672	0.011	0.275
LSD	ns§	ns	475	ns	329	ns
			Depth 30 to 60 cm			
Fertilizer high	915	865	777	857	969	1033
Fertilizer low	734	912	926	883	1029	942
Manure high	859	878	826	908	965	1030
Manure low	859	768	651	799	848	919
Control	915	843	1078	905	987	1037
P value	0.843	0.836	0.277	0.972	0.722	0.717
LSD	ns	ns	ns	ns	ns	ns
			Depth 60 to 90 cm			
Fertilizer high	570	486	756	583	544	632
Fertilizer low	519	502	455	457	551	593
Manure high	575	508	447	532	568	675
Manure low	558	377	482	567	515	609
Control	570	485	528	568	516	577
P value	0.901	0.559	0.002	0.124	0.761	0.862
LSD	ns	ns	125	ns	ns	ns

† Fertilizer rates were 90 and 179 kg N ha^{-1} yr^{-1} for the low and high levels, respectively.
‡ Three-year average manure rates were 236 and 459 kg total N ha^{-1} yr^{-1} for the low and high levels, respectively.
§ Not significant.

total NO_3–N than $^{15}NO_3$ leaching losses. Exchange of manure ^{15}N with soil N will have an effect of lowering $^{15}NO_3$–N concentrations until equilibrium is reached. In addition, although we did not follow $^{15}NO_3$–N changes with time, it is clear that the more frequent the manure applications, the greater the soil NO_3–N increase. It is realistic to infer that manure could impact N leaching in the future, especially on plots continuously manured.

Total Soil Nitrogen

Total soil N increases due to manure application as measured by ^{15}N enrichment at the end of the third cropping season are presented in Fig. 3. Values ranged from 52 to 373 kg N ha^{-1} to a depth of 90 cm. The N increase in plots receiving manure every year (1998 through 2000) was 373 kg ha^{-1}, considerably more in line with the cumulative manure N input (743 kg ha^{-1}), compared with 2000 kg ha^{-1} estimated by unlabeled N (Fig. 1).

The highest increase in soil ^{15}N was found in plots that received manure for three consecutive years and every other year. This trend was observed for the total sampled soil profile (0- to 90-cm), as well as across each of the 30-cm incremental depths.

Soil Nitrogen Balance

Manure and fertilizer N applications, crop N uptake, and differences in total soil N at the onset and end of the 3-yr trial were used to compute soil N balances for each treatment. According to the difference method

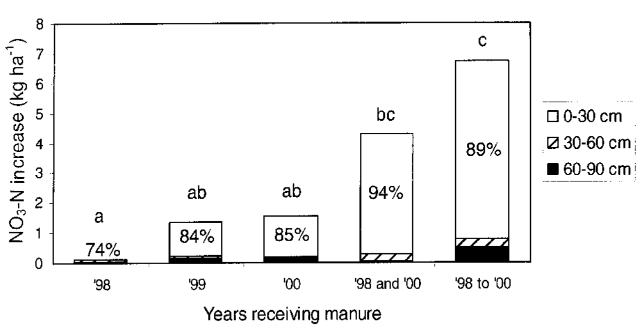

Fig. 2. Soil NO_3–N increase due to manure application frequencies as estimated by ^{15}N measurements in south-central Wisconsin, 2000. Numbers above each bar represent the percentage of recovered $^{15}NO_3$–N present in the top 30 cm only. For NO_3–N increase in the 0- to 90-cm depth, bars with the same letter are not significantly different at the 0.05 level.

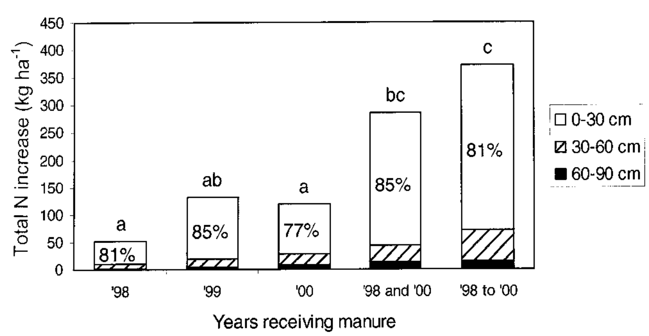

Fig. 3. Total soil N increase due to manure application frequencies as estimated by ^{15}N measurements in south-central Wisconsin, 2000. Numbers above each bar represent the percentage of recovered total soil ^{15}N present in the top 30 cm of soil only. For total N increase in the 0- to 90-cm depth, bars with the same letter are not significantly different at the 0.05 level.

Table 5. Total N applied and recovered in harvested corn for treatments receiving repeated fertilizer and manure applications in south-central Wisconsin, 1998 to 2000.

Treatment	Manure N applied 1998	1999	2000	Crop N uptake 1998	1999	2000	Crop N recovery†
	kg ha^{-1}						%
Fertilizer high	179	179	179	390	236	217	46
Fertilizer low	90	90	90	301	222	210	51
Manure high	388	501	489	261	194	226	6
Manure low	194	250	233	216	197	215	5
Control	0	0	0	246	166	185	–

† Cummulative, as a percentage of applied N, as calculated by the difference method (Eq. [1]).

(Eq. [1]), only 5 to 6% of total applied manure N was recovered in total corn dry matter by the end of the third cropping season, vs. 46 to 51% for applied fertilizer N (Table 5). Lower apparent recovery of manure than fertilizer N was mostly due to greater amounts of manure than fertilizer N applied, and to a lesser extent, somewhat lower corn N recovery in plots amended with manure than fertilizer. The apparent relative amount of fertilizer and manure N recovered in the soil (Eq. [2]) was two to seven times greater than N applications. Such gains in total soil N over and above N applications cannot be considered a rational result. The most logical explanation for these high estimates of soil N increases is that the fertilizer and manure N inputs represented only 1 to 10% of the basal soil N levels, which is less than the accuracy of the sampling and analytical methods used. The sampling and normal field variation likely further increased the uncertainty of the determinations.

Using ^{15}N measurements, from 13 to 22% of applied manure N was recovered in the harvested corn during the 3-yr study period (Table 6). Manure application interval had no significant effect on cumulative N recovery by corn. Sørensen et al. (1994) found that after two cropping seasons, 24% of applied ^{15}N was recovered by barley (*Hordeum vulgare* L.) and ryegrass (*Lolium perenne* L.), although a small fraction of recovered N was present in roots. A higher ^{15}N recovery (28%) after two barley crops was found by Thomsen et al. (1997). Still a higher cumulative ^{15}N uptake (56%) by barley across 2 yr was found by Jensen et al. (1999). In this last study, barley was undersown with ryegrass, and the soil was continuously cropped; therefore, it was reasonable to recover a higher percentage of ^{15}N than when the soil was fallow for some periods, as in our study. In addition, all these experiments were performed in small lysimeters or confined plots (30- to 100-cm diam.) where manure was immediately covered with soil, minimizing NH_3 volatilization losses. Further differences might arise from the crops grown, manure type (sheep or chicken), and soil conditions.

In plots receiving manure every year, 19% of applied ^{15}N was accounted for in harvested corn. The lower recovery of unlabeled manure N in crops (5%, Table 5) is probably due to the high N uptake in control plots throughout the experiment, which is subtracted out using the difference method (Eq. [2]). Indeed, when crop N uptake in control plots is greater than in a treatment plot (i.e., 1998 low manure applications), a negative manure N recovery is obtained. Without question, the ^{15}N method provided a much better direct estimate of manure N uptake by corn.

Approximately one-half to two-thirds of applied manure N was recovered in soil (0 to 90 cm), with one exception. Only 24% of the manure ^{15}N applied in 1998 was recovered in soil. Nitrogen recovered in the soil probably included slowly-decomposing and recalcitrant fractions of manure (undigested feed N in feces, which accounts for ≈20% of fecal N excreted by dairy cows; Powell and Wu, 1999), and manure N that was incorporated into new microbial biomass. Although our experiment did not allow us to corroborate this, it would be possible to label individual manure pools with ^{15}N and follow their fate within the soil-crop system.

The effects of year and frequency of manure application on the amount of applied ^{15}N recovered in the soil were not statistically significant. An average of 80% of the total ^{15}N measured in the soil was present in the 0- to 30-cm depth, with ≈13 and 6% in the 30- to 60- and 60- to 90-cm depths, respectively. This suggests either relatively little downward movement of applied manure N, or that leached N may have moved out of the 0- to 90-cm layer. Nitrogen increases below 30 cm must have come from NO_3–N movement to lower soil depths and subsequent immobilization, mixing by soil fauna, or pos-

Table 6. ^{15}Nitrogen recovery to a soil depth of 90 cm for different manure treatments in south-central Wisconsin, 1998 to 2000.

Years receiving manure	N recovery† Soil 0–30 cm	30–60 cm	60–90 cm	0–90 cm	Crop	Total	Unaccounted for
	%						
1998 to 2000	40 (11.7)‡	8 (2.4)	2 (0.2)	50 (12.8)	19 (3.1)	69 (15.5)	31 (15.5)
1998 and 2000	52 (19.6)	6 (1.6)	3 (0.7)	62 (21.6)	13 (3.1)	75 (24.4)	25 (24.4)
1998	18 (9.0)	4 (1.4)	1 (0.6)	24 (10.3)	17 (2.0)	41 (11.7)	59 (11.7)
1999	40 (7.6)	5 (0.9)	2 (0.4)	47 (8.5)	21 (2.1)	67 (9.7)	33 (9.7)
2000	37 (8.9)	7 (2.0)	4 (0.9)	48 (11.8)	22 (7.5)	70 (5.8)	30 (5.8)

† Cumulative recovery at the end of the third year, as a percentage of excess ^{15}N applied.
‡ Standard errors are given in parentheses.

sibly from roots. Depth differences in ^{15}N recovery were statistically significant ($P < 0.001$), with highest recoveries obtained from the top 0- to 30-cm depth (38% of applied ^{15}N). No differences in ^{15}N recovery were observed between the 30- to 60- (6%) and 60- to 90-cm (2%) depths. Other studies have recovered a greater proportion of applied ^{15}N in soil, such as 76 to 83% from the 0- to 10-cm depth, and 11 to 19% from the 10- to 45-cm depth (Sørensen et al., 1994). Sørensen and Jensen (1998) recovered 77.5% of applied manure (sheep) ^{15}N from 0- to 15-cm depth and 1.6% from 15 to 30 cm. Jensen et al. (1999) recovered 39% of applied sheep manure ^{15}N in the top 25 cm of soil. As discussed previously, higher N recoveries in these studies might have been due to rapid manure incorporation and subsequent lower volatilization losses. For all studies where ^{15}N was measured at different depths, most of it was recovered in the top 10 to 15 cm. Sommerfeldt et al. (1988) also found manure to affect total N and organic matter to a depth of 30 cm only.

In plots receiving manure in 1998 only, the highest amount of ^{15}N (59%) could not be accounted for. Actual N losses were not measured, but a longer period of time elapsed between spreading and incorporation of the manure in 1998 ($\approx$20 h) than during the other two study years ($\approx$2 h), possibly allowing for greater NH_3 volatilization. Our measurements do not seem to indicate much leaching (Table 2 and Fig. 2), although some losses via this pathway may have occurred. Unaccounted-for ^{15}N (36% on average) was probably lost mainly through NH_3 volatilization and denitrification. Denitrification losses have been estimated to range from 0.2 to 7.1% of incorporated dairy manure N (Goodroad et al., 1984; Lessard et al., 1996) with usually higher losses (up to 26%) for slurries (Thompson et al., 1987; Paul and Zebarth, 1997a,b). Ammonia volatilization losses as high as 61 to 99% of the N applied as NH_4 have been measured for broadcast manure in 5 to 25 d (Lauer et al., 1976). Liquid dairy manure can lose 24 to 33% of its NH_4–N in 6 to 7 d after being disked (Beauchamp et al., 1982). Up to 40% of NH_4–N can be volatilized, even within a few hours (Meisinger and Jokela, 2000).

It is apparent that soil N balances based on ^{15}N measurements are less variable and, therefore, likely more reliable than those based on unlabeled N. The use of ^{15}N labeled dairy manure provided direct measurements of manure N in the crop and soil system. As long as manure ^{15}N enrichment is high enough to be detected, analyses are more precise. The drawback of using ^{15}N-labeled manure is that the experimental setup and the analyses are more expensive and time consuming, and require significantly more careful sample preparation.

CONCLUSION

Our study showed that three successive annual manure or fertilizer applications did not affect NH_4–N but increased NO_3–N levels in soil. Manure treatments had a greater effect on the topsoil, whereas fertilizer did the same in deeper soil layers. There was a clear increasing trend in NO_3–N buildup with time for manure treatments, which may result in leaching problems in the long term. Repeated manure applications also increased soil total-N levels.

Use of ^{15}N-enriched manure confirmed the trends in vertical distribution of soil N observed with unlabeled manure. It also showed that increases in soil N were higher in plots receiving more frequent or recent manure additions. Clearly, ^{15}N-labeled manure provided more accurate measurements of total soil N and estimates of soil N balance. Using ^{15}N-labeled manure, 18% of applied N was recovered in harvested corn. Approximately 46% of applied manure ^{15}N remained in soil, and 36% could not be accounted for. Most of these losses were probably due to NH_3 volatilization between the time of manure field application and soil incorporation, and denitrification throughout the cropping season. Most of the N remaining in soil (an average of 82% of that recovered) was present in the top 30 cm, irrespective of frequency of manure application.

The results obtained in this experiment showed that, although more costly and time-consuming, ^{15}N studies can be a valuable tool in dairy manure research. This method proved to be a better approach than unlabeled manure to study the fate of manure N within the soil-crop system.

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